EFFECT OF ECOLOGICAL FACTORS ON THE LEAF EPIDERMIS OF SPECIES SOLANUM

M. JUHÁSZ

Botanical Institute of the Attila József University, Szeged
(Received September 30. 1966.)

The tissue structure of leaf epidermis is examined by several papers.

According to the majority of scientists, some of the tissue elements of epidermis maintain conservatively the features of species being thus useful as a systematic mark at diagnostificating the single species.

ZÖRNIG and WEISS (1925) consider the relation of the epidermis cells and the palisade parenchyma cells below them to be constant; WALLIS (1946), on the other hand, mentions the vein-islet number of leaf, the stomatal ratio and stomatal index as marks of diagnostical importance.

SÁRKÁNY and RIEDEL (1951) emphasize length and width of the guard cells of stomata, resp. their quotient, the stomatal size; and MARÓTI (1965), in the course of his investigations on the leaves of Pteridophytes, found the ratio of perimeter per surface of the epidermis cells as well as the percentage distribution of the subsidiary cells of stomata to be constant.

The above-mentioned qualities are quantitatively measurable characteristics of the epidermis. From the formal, qualitative qualities of the epidermis there are known the shape of epidermis cells, the microstructure of their cell-walls, the forms of trichomes, the way of the origin of stomata, the quality of silica-cells as marks for determining the species (LINSBAUER, 1930; SÁRKÁNY and FILLÓ, 1951; MARÓTI, 1965).

Recently we have found more and more allusions in the scientific literature to the fact that also the epidermis of plants reacts sensitively to the environmental effects from outside, and the several ecological factors (light, temperature, soil conditions) can change the stucture of the skin-tissue-system, as well (SINNOTT, 1960; SIMON—WOLCSÁNSZKY, 1964; SHANKS, 1965).

The present paper is treating of the examination of the effect of the external environment on the leaf epidermis.

In the course of our experiments we have wanted to get an explanation whether or not the quantitative epidermal features of the specimens of a species *Solanum*, developed in different surface soil relations, show up differences significant enough to unfit the epidermal quality in question for diagnosticating the species or to make questionable its reliability.

There were measured the following epidermis values: the relative number of stomata, stomatal index, length and width of stomata and their size (stomatal

length per width). For valuation of results the mathematic-statistical method was applied, highly increasing in that way the objectivity and exactness of the valuation of our results.

Materials and methods

Some species of *genus Solanum*, showing up a great abundance of species, have been used for being examined. *S. dulcamara* L. and *S. nigrum* L. are growing wildly in Hungary, while *S. laciniatum Ait.*, *S. giganteum* JACQ. *S .sodomaeum* L. are cultivated experimentally because of their great steroid-alkaloid-glycosid content.

For examining the plant-ecological effects of the different soil relations, specimens of *S. dulcamara* L., of *S. nigrum* L., and of *S. laciniatum* AIT., grown in five-five different soils were gathered in. The croplands were chosen in the way that two-two specimens were ingathered at all the three species from places of an extreme soil.

There were always selected plan specimens in flower and the leaf samples were taken from them. From every soil the leaf samples were taken from the middle leaf region of the stalks of three-three plant specimens. The gathered leaves were fixed in JUEL'S mixture, boiled in SCHULZE'S macerating solution, and after rinsing they were stained by EHRLICH'S haematoxylin-vesuvin double staining. The so prepared epidermis preparations from the upper and lower surfaces were conserved in glycerin-gelatin.

For determining the number of epidermal cells and stomata, the preparations were projeced from a microscope placed under the drawing table. The length and width of stomata were measured by an ocularmicrometer. At every preparation we measured 30—30 fields of sight, i. e., 90—90 data in every cropland. For valuating the results in biometric way, we prepared the curve of distribution of the several groups of data. These curves showed forms being very similar to GAUSS'S curves; the data of our measures have, therefore, been of a very normal dispersion.

The valuation of results was carried out by variancy analysis. At preparing the basic tables of variancy analysis, we formed three-three lines and five-five columns.

In the lines the arithmatic means of the 30—30 measurement-data of three-three preparations made from specimens grown in the same cropland were registered.

The five-five columns contained epidermis data corresponding to the plants of the five different soils.

Thus we have obtained basic tables with fifteen headings.

Then the variancy tables were constructed and the corresponding calculations carried out.

In the course of the significancy examinations, the validity of our conclusions from the numerical data of our examinations was controlled by F- (FISHER) and t- (STUDENT) tests.

It was settled by F-test whether or not the values of the numerical differences between the different soil means are greater than the means of epidermis values of the specimens of the same soil on a $P=5$ p. c. probality level.

We calculated by t-test the differences inside which the values may only be attributed to test failures caused by the differences of sample selection.

The SD_5 per cent values are made known at the description of results.

Results

(1) Determination of the site of sampling

Before examining the effects of the environmental factors, we considered to be necessary to determine the site of the epidermal excoriation. We know ZALENSKY'S statement (1904) aceording to which the number of stomata is gradually growing as we advance from the lower part of stalk to the top of that. Accordingly, it is not indifferent from which nodules of the stalk of a plant the leaf samples are obtained. Some researchers found differences between the stomatal

numbers of the basis, middle, and apex of the leaf sheet even inside the single leaves. According to SLAVIK (1963), there are significant differences between the stomatal numbers of the basal and apical parts of the tobacco leaves. SIMON—WOLCSÁNSZKY (1964) has observed a decrease of the stomatal number characterized by a regressive straight, advancing from the basis to the apex of the leaf sheet of yellow corn.

For these examinations we have selected the *S. sodomaeum* L. of small stature and of small leaf size the. *S. laciniatum* AIT. of middle stature and of middle leaf size, and the *S. giganteum* JACQ. of tree-stature and of large leaves. Leaves were gathered in from the nodules of the lower, middle, and upper parts of three-three flowering specimens of all the three species. There were excoriations prepared from the lower surface of leaves at the basis, leaf middle, and apex of the leaves gathered in. On every preparation the number of stomata of a field of 60 sq. mm was valuated statistically. The results are demonstrated in Figs. 1/a and 1/b.

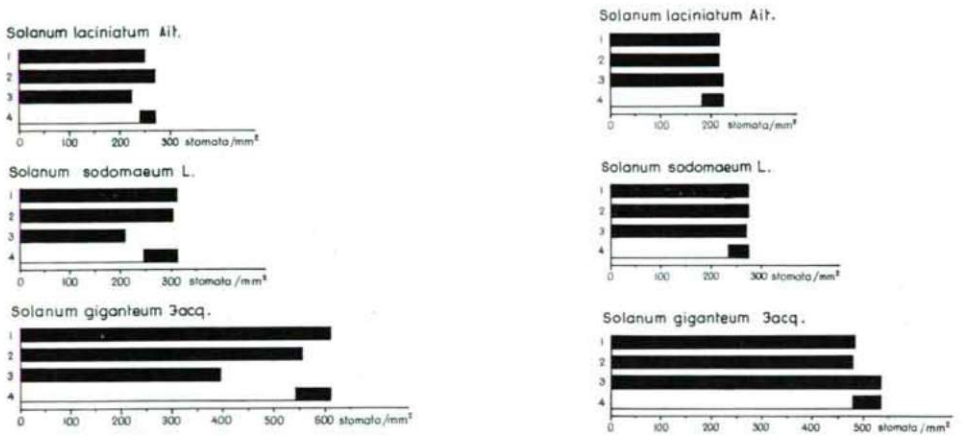

Fig. 1. a. Stomatal number of the leaves of stalk arising different heights (lower epidermal surface).
1 = upper leaf region;
2 = middle leaf region;
3 = lower leaf region;
4 = SD_1 p. c.

b. Somatal number of different parts of the leaf sheet (lower epidermal surface).
1 = leaf apex;
2 = leaf centre;
3 = leaf basis;
4 = SD_5 p. c.

It can be ascertained that:

(a) At the leaves arising from different heights of the stalks of species *Solanum,* the turn of the number of stomata follows roughly ZALENSKY's rule, *i. e.*, the stomatal number is the smallest at leaves in the lower part of stalk, going towards the apex, however, it grows gradually. This can be seen the most clearly

in the case of the *Solanum giganteum* JACQ. of tree stature, while in the case of the other two species only the stomatal number of leaf region differs (even on SD_1 per cent level) from the number of stomata of the other leaf regions. It is advisable to take a leaf sample from the middle part of stalk as the stomatal number of the middle leaf region gives the arithmetic mean. We have possibly to select leaves of identical position, node for every comparison.

(b) At the formation of stomatal number inside the leaf sheet it can be noticed that the difference between the single parts of leaves is but minimal. Only the stomatal number of the leaf basis of *Solanum giganteum* JACQ. with 25—30 cm life size differs significantly on SD_5 per cent. Regarding also the data of SLAVIK (1963) and SIMON—WOLCSÁNSZKY (1964), we may draw the conclusion that the differences between leaf parts are to be taken into consideration chiefly in the case of leaves of large size. In that case, too, it is the best to prepare the excoriation from the middle of leaf sheet. We consider as important to be noticed that the two sides of the main vessel of leaf are no reflections of each other in a mirror; it is therefore advisable to take samples from both sides of the main vessel.

(2) The effect of differring cropland conditions on the leaf epidermis

During the epidermis examination of the plants of *S. laciniatum* AIT., *S. dulcamara* L., and *S. nigrum* grown in five-five different croplands the greatest differences were observed concerning the change of the epidermal cell number. The number of epidermal cells shows up great differences at species developed in different conditions. It can be observed that we can find much more epidermal cells on the lower epidermal surface than on the upper surface. That is the cause of being very few stomata on the upper surface, on the lower surface, however, many ones. The stomata are of *Cruciferae*-type (METCALFE and CHALK, 1950), therefore every stomata being surrounded by three-four subsidiary cells. Thus it can be understood why these cells are smaller than the epidermal cells of the upper surface. The ratio of the cells of the lower epidermal surface per those of the upper epidermal surface is, however, constant in every cropland. The formation of the relative stomatal number, stomatal index, stomatal length and width, as well as that of the stomatal size in different croplands can be observed in Figs. 2, 3, 4, 5 and 6. In the column diagrams, the white columns are showing the data of the upper epidermal surface, the black columns those of the lower epidermal surface. In every figure also the SD_5 per cent values are given, these being the values of the greatest differences still acceptable.

Also the number of stomata belonging to the unit of a leaf surface demonstrates great differences. Projecting differences are, however, to be found but at the species developed in extreme conditions, the data of the three-four similar croplands are close to one another. This is connected with the finding that the plants react upon the effect of the outer circumstances first of all by the size of cells; that however, may not be a dicisive factor, as proved by the change of stomatal index.

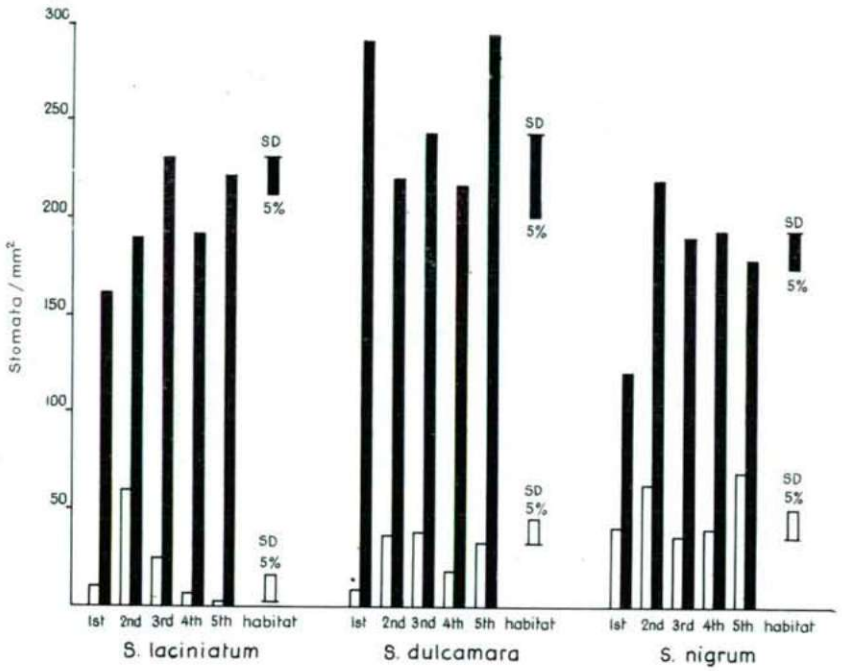

Fig. 2. Formation of the stomatal number at specimens grown in different croplands.

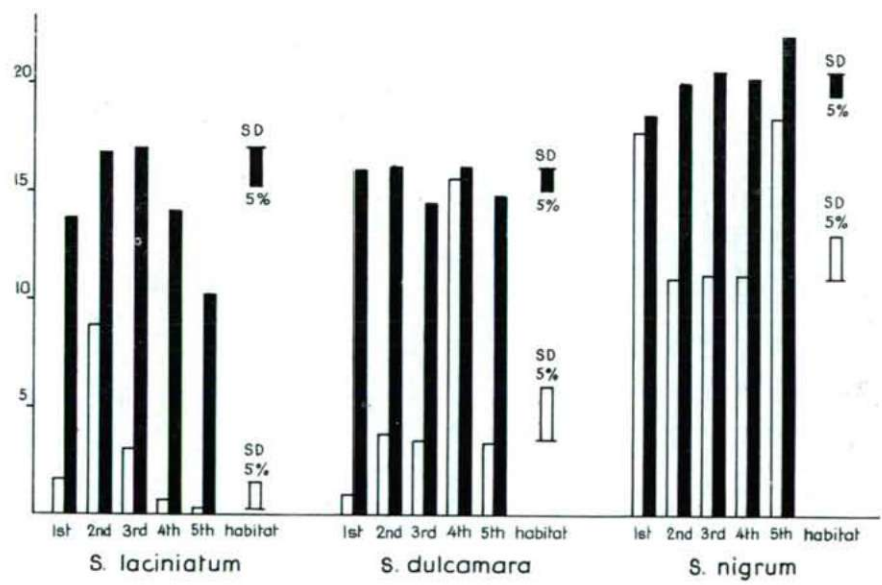

Fig. 3. Change of the stomatal index under the influence of environmental effects.

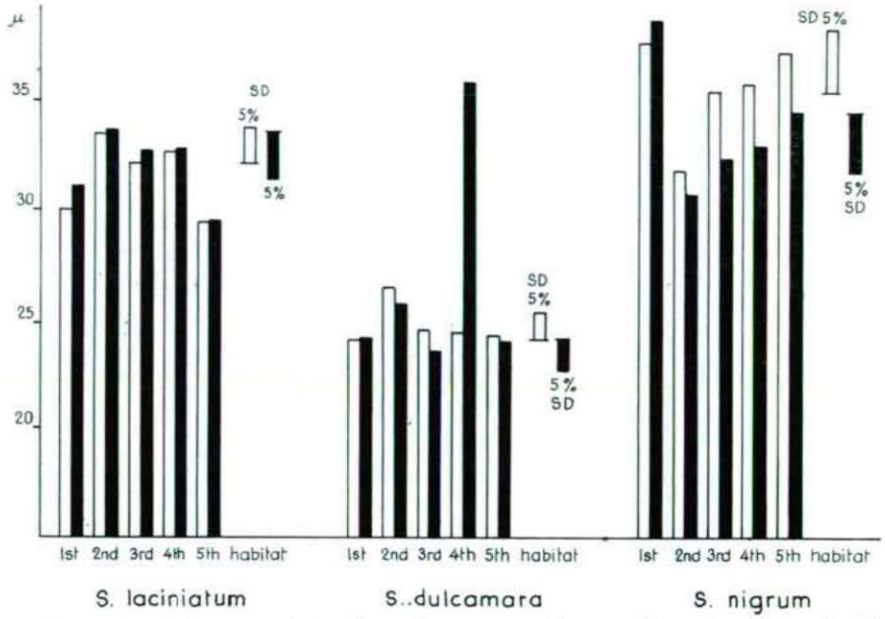

Fig. 4. Formation of the stomatal length on the upper and lower epidermal surfaces of *Solanum* leaves.

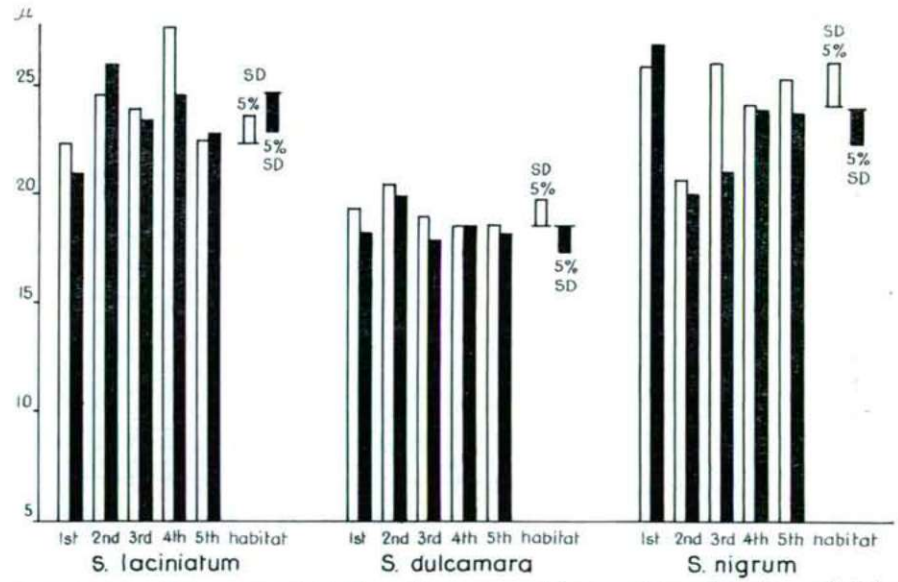

Fig. 5. Formation of the stomatal width on the upper and lower epidermal surfaces of *Solanum* leaves.

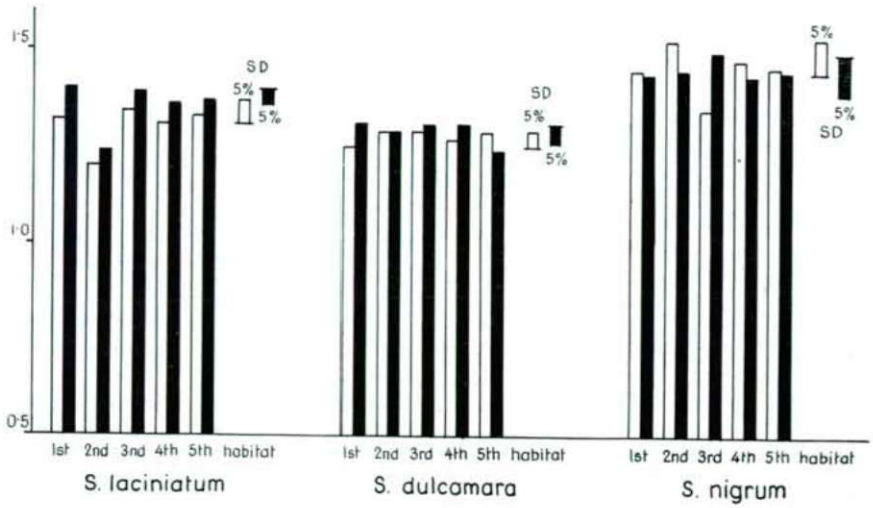

Fig. 6. Formation of the stomatal size on the upper and lower epidermal surfaces of *Solanum* leaves.

According to some authors, the ratio of epidermal cells and of stomata to each other is determined genetically. This means that even if the extent of stomata and epidermal cells changes, influenced by different ecological factors, their size increases or decreases proportionately, and therefore the stomatal index (stomatal number per number of epidermal cells plus stomatal number) cannot change.

It can be observed in Fig. 3. that the stomatal index shows differences similar to the stomatal number; between specimens developed in ecologic conditions similar to each other the difference is of lesser degree, the stomatal index of the leaves of individuals living under extreme condition differs, however, even on SD_1 p. c. level from that of other ones. The external circumstances exercise, therefore, a significant influence on the internal, genetical connections of the skin tissue.

Length and width of the guard cells of stomata change at the Solana living under different conditions in a smaller degree than the stomatal number and index; nevertheless, even these are not suitable to determine the single species with absolute certainty. It is obvious that even the guard cell data of the stomata of upper and lower surfaces are not agreeing thoroughly with one another; the difference of their sizes is, however, inside a probability level of 5. p. c.

The stomatal size (length per width of tomata) is a ratio, it gives a directing value concerning the shape of the guard cells of stomata.

It was indicated already by the values of F-test that there are no differences here between the values of the different soil data that could be valuated on SD_5 p. c. The stomatal size is, therefore, the most stable epidermal mark among the qualities examined until now.

Discussion

(1) It can be ascertained on the basis of valuation of the stomatal numbers measured in different regions of stalks and leaves that the middle of the leaf sheet obtained from the middle stalk part is suitable to be examined.

(2) The quantitative epidermis qualities of the species *Solanum* developed under different soil conditions show up differring changes if influenced by the environment. The stomatal size changes the least. The stomatal length and width, as well as the stomatal index and relative stomatal number undergo a more considerable change.

For diagnosticating the species, the stomatal size is the most suitable. The employment of the other epidermal marks is less reliable; it can be employed with succes only for species living under similar conditions. Genus *Solanum* is one of the genera with the highest species number, still developing. This is manifested in reacting to the effects of the external environment more sensitively as well, and is reflected also by the formation of some quantitative qualities of the leaf epidermis as emphasized above.

(3) At the analysis of the leaf epidermis on the basis of quantitative epidermal qualities we consider the employment of biometrical methods to be absolutely necessary.

Summary

We have examined the change of some epidermal qualities, measurable quantitatively, on the leaves of specimens of *Solanum laciniatum* Ait., *S. dulcamara* L., and *S. nigrum* L., grown in different croplands. It was ascertained that the external, ecological conditions had a considerable influence on the tissue structure of the leaf epidermis, making questionable the species-diagnostical value of the examined epidermal marks.

References

Linsbauer, K. (1933): Handbuch der Pflanzenanatomie. — Berlin.

Maróti, I. (1965): Vergleichende anatomische Untersuchungen an den Blättern der *Ophioglossaceae*. — Acta Biol. Szeged, *11*, 55—82.

Metčalfe, C. R.—Chalk, L. (1950): Anatomy of the *Dicotyledons*. — Oxford—Clarendon 2. 971.

Sárkány S.—Filló, Z. (1951): Kvantitatív mikroszkópi vizsgálatok a gyógynövények és hamisítványaik lomblevelén. — Ann. Biol. Univ. Hung. *1*, 107—119.

Sárkány S.—Riedel, L. (1951): Adatok a *Nicotiana*-fajok javaleveleinek anatómiájához. — Ann. Biol. Univ. Hung. *1*. 119—138.

Simon—Wolcsánszky E.—Molnáros I. (1964): Der Aufbau der Maisepidermis, die Veränderungen ihrer Spaltöffnungszahl unter der Wirkung der ökologischen Faktoren I. — Acta Bot. Ac. Sci. Hung. *10*. 351—368.

Sinnott, E. W. (1960): Plant Morphogenesis. — Mc. Graw-Hill New-York—Toronto—London 181—229.

Slavik, B. (1963): On the problem of the relationship between hydratation of leaf tissue and intensity of photosyntheses and respiration. — London.

Shanks, R. (1965): Differentiation in leaf epidermis. — Austr. J. Bot. Melbourne *13*. 143—151.

Wallis, I. (1946): Textbook of Pharmacognosy. — London.

Zalensky, B. P. (1904): Materiali kolichestvennoi anatomii razlichnih listyev odnikh i teh je rastenii. — Izv. Kievsk. Politeh. Inst. *4*.

Zörnig, H.—Weiss, G. (1925): Beiträge zur Anatomie des Laubblattes offizineller und pharmazeutisch gebräuchlicher *Compositen*-Drogen. — Arch. Pharm. Berlin, 451—470.